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Human Intrinsic Factor Secretion:
Immunocytochemical Demonstration of Membrane-
associated Vesicular Transport in Parietal Cells

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ABSTRACT   The human gastric parietal cell synthesizes and secretes intrinsic factor (IF) and
acid. In contrast to the cellular mechanisms of acid secretion, little is known about the
mechanisms of IF secretion. To elucidate these mechanisms we obtained gastric secretions and
sequential fundic biopsies from three subjects before and after pentagastrin stimulation (6
µg/Kg s.c.). IF was localized in the biopsies using an ultrastructural immunoperoxidase tech-
nique using a well-characterized, monospecific antibody to human IF. IF output was quantified
using a specific radioimmunoassay in concurrently obtained gastric secretions.

Before stimulation, IF was associated with tubulovesicles scattered throughout the cytoplasm
and with some in rough endoplasmic reticulum (RER). The tubulovesicles associated with IF
migrated to the periphery of the secretory canaliculi within 8 min of stimulation. IF was present
on secretory microvilli between 8 and 30 min when IF output in gastric juice was at its
maximum. The cessation of IF secretion coincided with the depletion of IF associated with
tubulovesicles. IF appeared in the perinuclear space and RER as the IF associated with
tubulovesicles was secreted. These observations indicate that IF secretion depends upon
membrane-associated vesicular transport and provides support for a membrane translocation-
fusion hypothesis to explain the morphologic changes that occur in the parietal cell during
secretion.

It is generally accepted that the parietal cell is the source of
both acid and intrinsic factor (IF) in man (1). In contrast to the
rapidly expanding knowledge about the cellular mechanisms
of acid secretion by the parietal cell (2, 3), little is known about
IF secretion. Currently available information about IF secre-
tion is limited to the measurement of IF within gastric secre-
tions. In in vivo human studies, all substances that stimulated
acid secretion, such as histamine (4), methacholine (5), and
gastrin (6), also stimulated IF secretion; and interventions that
suppressed acid secretion, such as vagotomy (7) and the ad-
ministration of secretin (6) or cimetidine (9), also suppressed
IF secretion. Characteristically, IF secretion is maximal in the
first 15–20 min after stimulation, and decreases rapidly at a
time when acid secretion is still increasing. This pattern of
secretion is present regardless of whether a bolus (4, 6, 7, 9) or
a constant infusion (5, 8) of secretagogue is administered. It
was conjectured that the failure to continue to secrete was
secondary to the "wash-out" of some intracellular storage form

of IF as acid is being secreted (4, 7, 9). Recently, Kapadia and
Donaldson (10) measured the basal rate of secretion of IF from
organ cultures of rabbit fundus and found that it was dimin-
ished after applying acid to the explant's mucosal surface.
These investigators speculated that the rapid decline in IF
secretion seen in vivo after stimulation might also be caused
by acidification of the gastric mucosa, rather than exhaustion
of stored IF.

The pronounced morphologic changes that occur in parietal
cells following stimulation have been well documented. Quan-
titatively, there is a significant decrease in tubulovesicles and
an increase in surface membrane following stimulation (11–
14). The interpretation of these findings was that translocated
tubulovesicles provide the source for the increased surface
membrane of acid-secreting parietal cells (15). Definitive dem-
onstration of this translocation has been inferential.

We have recently reported (16) the immunocytochemical
localization of IF within nonstimulated parietal cells of human

gastric mucosa. Most IF was found associated with tubulovesicles scattered throughout the cytoplasm. Small amounts of IF were also present in rough endoplasmic reticulum (RER) found basal to the cell nuclei, as well as occasionally associated with membranes of the perinuclear space, Golgi complex, and multivesicular bodies. These observations, together with the known morphologic changes that occur in the parietal cell following stimulation, suggested that IF secretion might depend on the translocation of tubulovesicles to the apical surface.

Using our well-characterized (16), monospecific antibody to homogeneously purified human IF, we undertook an ultrastructural immunoperoxidase study of fundic biopsies sequentially obtained before and after stimulation of acid and IF secretion in order to obtain a more complete understanding of the intracellular events that occur during IF secretion.

MATERIALS AND METHODS
Experimental Protocol

Three volunteers (one male, two female, age 22–24 yr) who had no personal or family history of gastrointestinal disease were seen after a 12-h fast. They had not used alcohol, aspirin, or other drugs for 1 wk before the study, and had a normal Ivy bleeding time. After informed consent was obtained (Human Research Committee approval, July 1978), the posterior pharynx was topically anesthetized and a Quinton hydraulic biopsy tube (Quinton Instruments, Seattle, Wash.) and a Salem sump tube (Boyle-Midway Div. of American Home Products

Corp., New York, N.Y.) were passed into the stomach. The sump tube was fluoroscopically positioned along the greater curvature, and the biopsy tube was positioned slightly higher in the fundus. The subjects were made to lie on their left sides, and any retained gastric secretions were aspirated and discarded.

Gastric secretions were continually hand-aspirated through the sump tube and pooled into consecutive 15-min collections. After 30 min of basal collection, 6 µg/kg of pentagastrin (Peptavalon, Ayerst Laboratories, Div. of American Home Products Corp., New York) was injected subcutaneously, and four more 15-min collections were obtained. 1-ml aliquots of each collection were mixed with 1 ml of 1M Tris buffer (pH 7.4), coded, and frozen for later immunochemical determination of IF. The remainder of the aspirated secretions were kept refrigerated until the concentration of (H⁺) was determined. A fundic biopsy was obtained during the basal period, and at 3, 8, 15, 30, 45, and 60 min after pentagastrin stimulation. All biopsies were oriented mucosa-side-up on filter paper, placed in a picric acid–2% paraformaldehyde fixative (17) at 4°C, and processed as detailed under immunocytochemical protocol.

Sources of Proteins and Antisera

Homogeneously purified human IF (18) and immunochemically monospecific rabbit antihuman IF antisera (19) were obtained as described previously.

Antiserum to rabbit gamma globulin was raised in sheep by multiple intramuscular injections of rabbit gamma globulin. Antibodies specific to rabbit IgG were isolated by passage over an affinity column of rabbit gamma globulin bound to Sepharose beads (20). F᷂b' fragments of the isolated sheep antirabbit gamma globulin were made by pepsin digestion (21) and conjugated to horseradish peroxidase (HRPO). The conjugate was separated from impurities by previously described methods (22). Nonimmune serum was obtained from normal rabbits (NRS). All antisera were diluted in phosphate-buffered saline (PBS)—10% sucrose—1% bovine serum albumin.
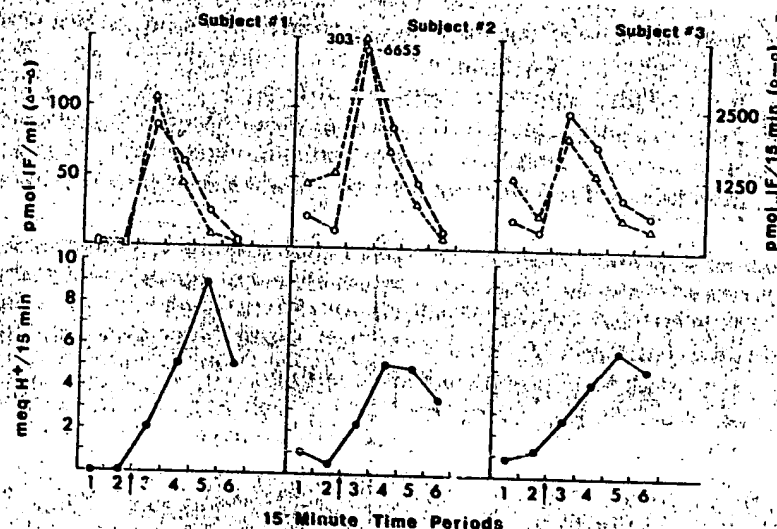

FIGURE 1 Intrinsic factor (IF) and acid secretion before (time periods 1 and 2) and after pentagastrin stimulation (arrow). Both IF concentration (pmol/ml) and output (pmol/15 min) were highest in the first poststimulation collection and then decreased while acid secretion was increasing in each subject.

TABLE I
Relative Proportion of Parietal Cell Organelles Containing IF

| Organelle | Basal | Time of biopsy (min) | | | | |
		3	8	15	30	45	60
Perinuclear membrane	+*					+++	++++
RER	+*	+	+	++	+++	+++	+++
Golgi apparatus	+/−	+/−	0	++	+++	++++	++++
Tubulovesicles (scattered)	++++		0	0	0	0	0
Pericanalicular tubulovesicles	+/−	++	++++	+++	++	0	+
Multivesicular bodies	+		++	+++	+++	+	++
Microvilli	+/−	+/−	++	+++	++	+++	+++
Basolateral membranes	0	0	0	0	+++	+	0

* Percentage of cells visualized in which any portion of these organelles contained immunoreactive human IF expressed to the nearest quartile (see Materials and Methods). 0 = 0%; +/− = <5%; + ≈ 25%; ++ ≈ 50%; +++ ≈ 75%; ++++ ≈ 100%

Immunocytochemical Protocol

The seven biopsies from each study were processed simultaneously using an indirect immunocytochemical protocol described in detail previously (16). Sections from each biopsy were reacted for 20 h with either a 1:10,000 dilution of antihuman IF, 1:1,000 NRS, or 1:10,000 antihuman IF preincubated with 1 μg IF. After washing, a 1:45 dilution of the sheep Fab' antirabbit IgG-HRPO was placed on the sections for 3 h. The sections were then sequentially placed in 2% glutaraldehyde, and 3-3'-diaminobenzidine—0.005% H_2O_2. At this stage some of the slides were processed for light microscopy (dehydrated in graded alcohols and xylene, a cover slip placed on the sections, and the slides coded). The remaining slides were processed for electron microscopy.

After osmication, embedding in Epon-Araldite (16), and coding, silver-gold sections were obtained at 1-μm levels through the entire depth of each block. The coded grids were viewed on a Philips 201 electron microscope at 60 kV after an entire study had been sectioned. Each block was evaluated as positive or negative for IF and whether ultrastructure was preserved. If positive for IF, the intracellular location of any immunogenicity was documented and recorded for each cell visualized (see below).

To further diminish possible selection bias all three studies were assessed, and the location of IF within the cells of each biopsy was recorded, before breaking the study code and relating the findings within each biopsy to their appropriate time period. This allowed comparison of multiple sections from individual biopsies, as well as biopsies from the same time period from different subjects.

Gastric Fluid Analysis

After measuring pH, each 15-min collection of gastric secretions was titrated to pH 7.0 with 0.1 N NaOH. The amount of titratable acidity was expressed as milliequivalents of H^+ per 15-min collection.

The coded aliquots of frozen gastric juice from all three studies were defrosted and the amount of IF determined using a specific radioimmunoassay (RIA) that has been described previously (23). This RIA differentiates between IF and non-IF B_{12} binding proteins. The amount of IF secreted per 15-min collection was expressed in terms of absolute output (pmol/15 min) and concentration (pmol/ml).

Intracellular Localization of Immunoreactive Human IF

Ultrastructural immunocytochemistry, as we have undertaken in this study, is not amenable to quantitation of IF within specific subcellular compartments. It became obvious after viewing several biopsies, however, that there were dramatic differences in the subcellular location of IF. Therefore, in order to express these differences and compare different subjects, the location of IF within specific cellular compartments was recorded for all intact parietal cells present at the mid-level (third grid) from each biopsy. The number of cells counted from each biopsy was 20-30, with a total number of 90-110 per time period. If any portion of a specific organelle (i.e., part of the perinuclear membrane) was stained, it was charted as positive. These counts were expressed as the percentage of cells visualized, to the nearest quartile, that contained a specifically stained organelle. Our definitions of subcellular compartments were:

(a) RER: Tubular structures that had a granular appearance. Ultimately, counterstaining with uranyl acetate and lead citrate identified polyribosomes on these structures when unstained (Fig. 1). Note was made whether the RER was limited to the basal portions of the cell or scattered throughout the cytoplasm (as described by Rubin [24]).

(b) Golgi apparatus: smooth, tubular, stacked membranous structures. Unstained Golgi complex (although present, as later seen when sections were counterstained) could not be seen.

(c) Perinuclear membrane: The percentage was derived as percent of nuclei with staining (because some cells had no nuclei at that level).

(d) Multivesicular bodies: Round-ovoid, smooth-membraned organelles containing small membranous structures. If any portion of this body was stained, it was considered to be positive.

(e) Microvilli: Any portion of the surface membrane abutting on the intracanalicular space or apex of the cell.
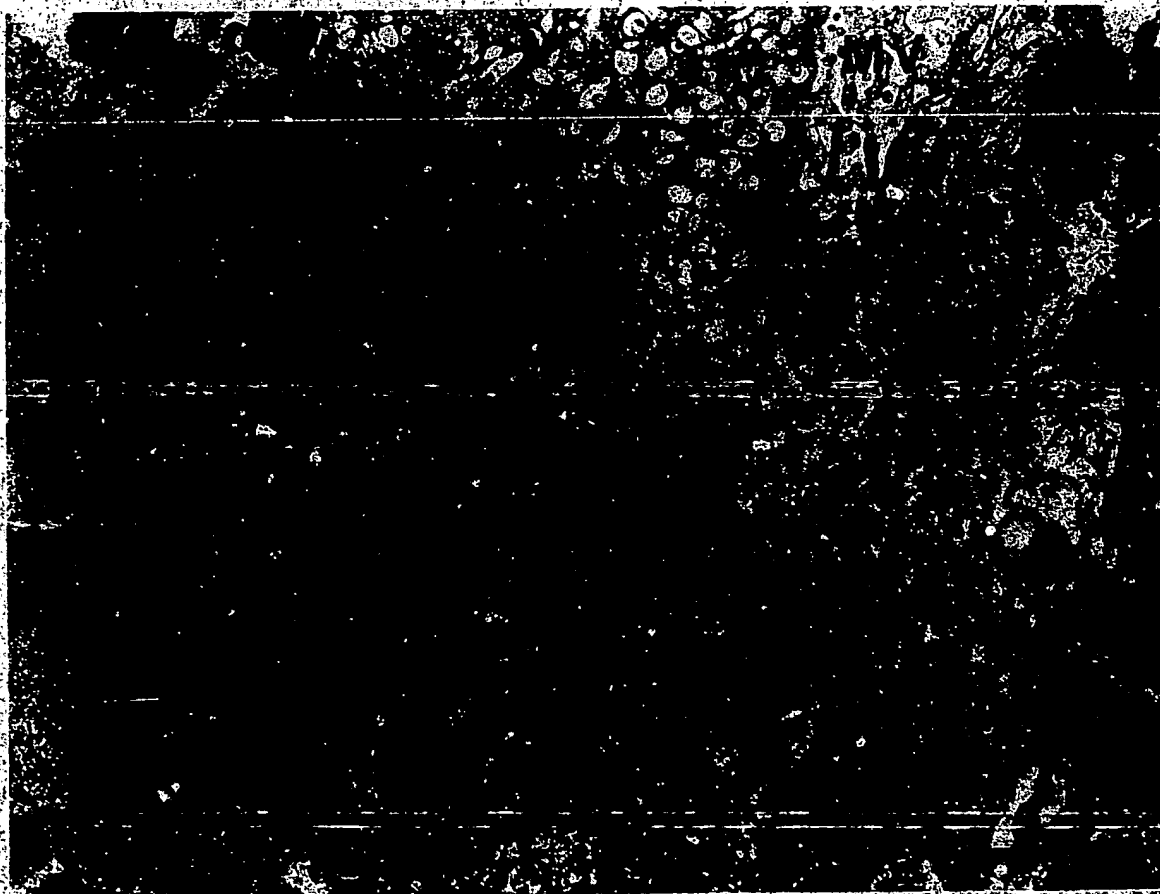

FIGURE 2 Electron micrograph of parietal cell in biopsy from basal period, in which antihuman IF has been adsorbed with IF, and no IF immunoreactivity is present. Rough endoplasmic reticulum (*RER*), nucleus (*N*), mitochondria (*M*), multivesicular body (circled, *MVB*), scattered prominent tubulovesicles (*TV*), and blunted microvilli (*MV*) within intracellular canaliculi (*IC*) are visible. ×20,000. Bar, 1 μm.

FIGURE 3. Electron micrographs of parietal cells in biopsy from basal period reacted with antihuman IF. (a) IF is present on scattered tubulovesicles (small arrows), RER, and multivesicular body (large arrow). Abbreviations as in Fig. 2. ×11,000. Bar, 1 μm. (b) Higher magnification showing IF on RER (small arrow) and Golgi (white arrow). ×50,000. Bar, 0.2 μm.
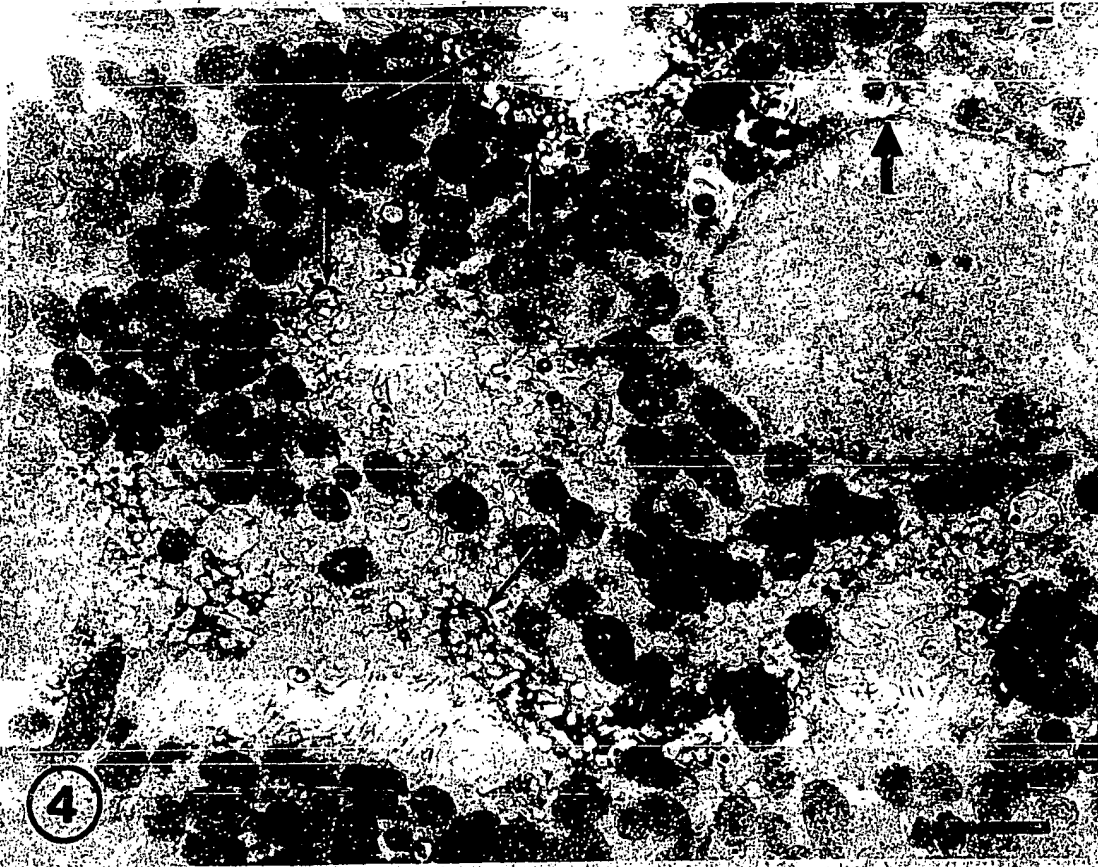
FIGURE 4 Parietal cell from biopsy taken 3 min poststimulation reacted with antihuman IF. Tubulovesicles containing IF (small arrows) are now aggregated around intracellular canaliculi and the apical surface (*TJ*, tight junction). Perinuclear membrane containing IF (large arrow) is present. Microvilli are not stained. ×15,000. Bar, 1 μm.
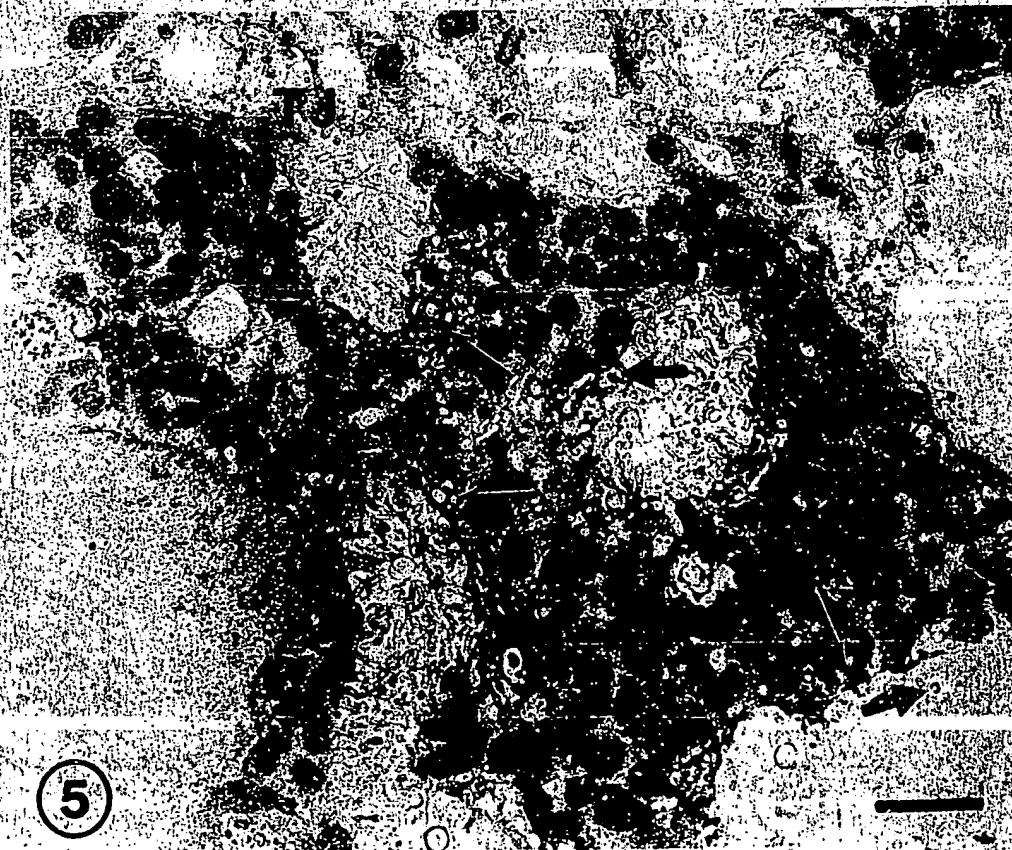
FIGURE 5 Parietal cells from 8-min poststimulation biopsy, reacted with antihuman IF. Pericanalicular and apical distribution of IF (small arrows) is evident. In contrast to the 3-min biopsy (Fig. 4), microvilli are now stained (large arrows). ×13,500. Bar, 1 μm.

FIGURE 6 Section from 15-min poststimulation biopsy, reacted with antihuman IF. Prominent pericanalicular tubulovesicle (*TV*) and microvillar (small arrows) staining for IF. ×32,000. Bar, 0.5 µm.

(*f*) Tubulovesicles (TVs) divided into three categories.

   (1) Scattered—positive TVs present in random distribution throughout the cytoplasm.

   (2) Pericanalicular—only given if essentially all positive TVs within a given cell were arrayed around the intracanalicular space and the apical surface.

   (3) Absent.

RESULTS

Gastric Analysis

The rates of secretion of acid and IF (Fig. 1), after a pentagastrin stimulus, are comparable to those in previous reports (6). Total IF output and concentration reached a peak within the first 15-min postpentagastrin collection, and then rapidly declined at a time when acid secretion was increasing. The basal pH varied between 2.1 and 7.0, whereas the pH of all poststimulation collections was <2.

Light Microscopy

When the sections prepared for light microscopy were read blindly, no specific differences could be ascertained in the distribution of IF in parietal cells between the basal and any of the poststimulation biopsies. There were no positive sections that lacked immunoreactive IF or negative control sections that contained IF.

Ultrastructural Immunocytochemistry (Table I)

After the study code was terminated and data from the three studies were collated, it was found that (*a*) all control sections had no IF immunoreactivity (Fig. 2), (*b*) multiple sections from any one biopsy specimen had shown the same distribution of IF, and (*c*) the location of IF within biopsies from the same time period in different subjects was qualitatively the same.

Basal Period

The placement of IF within the biopsies obtained before stimulation was identical to that found in our previous report (16). The majority of immunoreactive IF was associated with tubulovesicle membranes scattered throughout the cytoplasm (Fig. 3 a). Some IF was also found in RER and Golgi complex (Fig. 3 b) confined to basal portions of cells, as well as portions of perinuclear and multivesicular body membranes.

Postpentagastrin Stimulation

3 min: In about one-half of the observed cells, tubulovesicles associated with IF were found to be aggregated around the periphery of the intracellular canaliculi (e.g., pericanalicular) and apical surface membranes (Fig. 4). The distribution of IF associated with other intracellular organelles was unchanged from the basal biopsies.

8 min: The change from scattered to pericanalicular tubulovesicles was universal and in one-half of the cells IF was located on microvillar membranes (Fig. 5).

15 min: Essentially all the cells in the 15-min biopsies had canalicular or apical microvilli that had associated IF (Fig. 6), whereas the prevalence of pericanalicular tubulovesicle IF diminished. Although clearly associated with the surface membrane of microvilli, IF was also localized to separate membranous structures within microvilli. This was best demonstrated when microvilli were cross sectioned (Fig. 7, a and b). In a small portion of cells, RER associated with IF was no longer
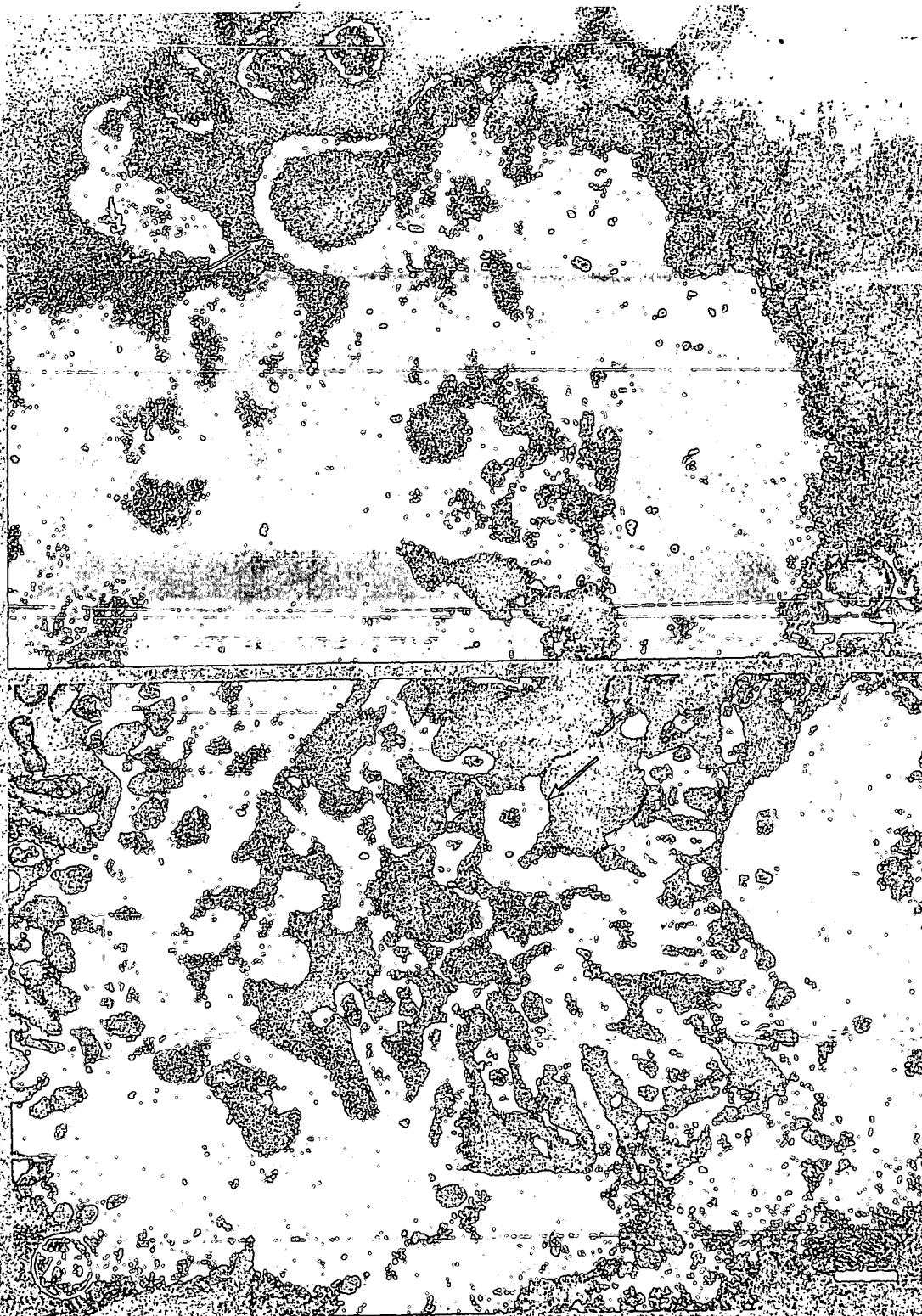
FIGURE 7 Details of canalicular lumen from 15-min poststimulation biopsy, reacted with antihuman IF. (a) Tubulovesicle containing IF (arrow) apparently pushing into the intracanalicular space. This could lead to a microvillus with a central transparency. ×66,500. (Bar, 0.2 µm.) (b) Multiple microvilli with IF on surface membrane, but also a cross-sectioned microvillus (arrow) with "doughnut" appearance and IF on both inner and outer membranes. ×50,000. Bar, 0.2 µm.

restricted to the basal part of the cell, but was scattered throughout the cytosol.

50 min: Two distinct populations of cells were identified in the 30-min biopsies. In one-half of the cells, RER containing IF was interspersed throughout the cytoplasm, and the peri-

nuclear membrane was densely stained. Commonly, in these same cells only a small amount of microvillar or tubulove-sicular IF was identifiable (Fig. 8). The remaining cells had a distribution of IF similar to the cells in the 15-min biopsies. Commonly, multivesicular bodies with associated IF (both
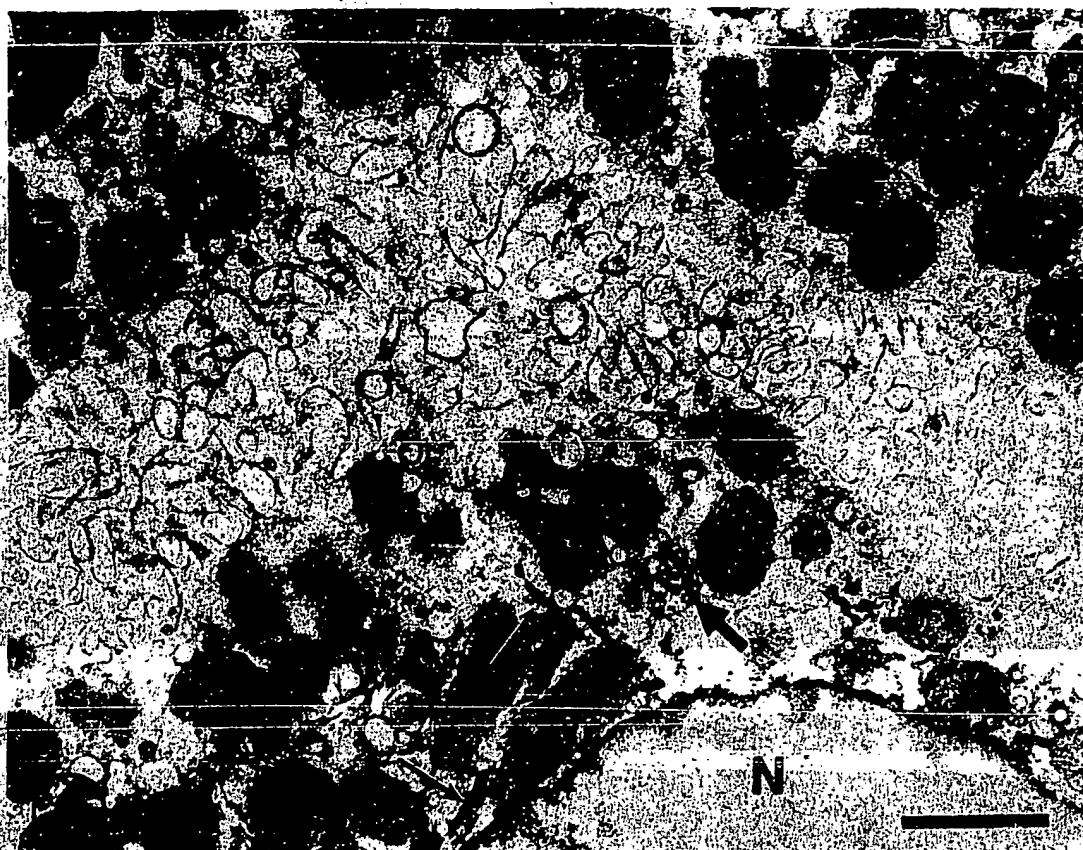
FIGURE 8   Section from 30-min poststimulation biopsy, reacted with antihuman IF. In contrast to earlier biopsies, microvillar and tubulovesicle IF is minimal. Prominent scattered RER (small arrows), perinuclear membrane, and multivesicular body (large arrow) contain IF. N, nucleus. ×20,000. Bar, 1 μm.

FIGURE 9   Section from 15-min poststimulation biopsy, reacted with antihuman IF. The outer membrane of a multivesicular body (MVB) contains IF and is continuous with a tubulovesicle-like structure abutting on the intracanalicular (IC) surface membrane. Small membranous structures within the MVB also contain IF. ×72,000, Bar, 0.2 μm.

surface membrane and intraorganelle vesicles) were positioned near the perinuclear space or the microvillar surface. Rarely, the surface membrane of a multivesicular body associated with IF was apparently continuous with pericanalicular tubulovesicular membrane (Fig. 9).

45 min: Microvilli and tubulovesicle staining had diminished, and perinuclear membrane, RER, and multivesicular bodies were the most commonly stained organelles (Fig. 10). Counterstained control sections show ribosomes on the scattered RER (Fig. 11 a). The ribosomes are obscured by the immunoreactive IF in positive sections (Fig. 11 b). The difference between tubulovesicles and RER is apparent.

60 min: One-fourth of the cells had returned to a nonstimulated morphology with scattered tubulovesicles and basal RER that stained for IF (Fig. 11). Microvilli were more commonly stained (≅25%) than in nonstimulated cells. These biopsies could be clearly separated from the basal and 3-min specimens by the prominently stained scattered RER and perinuclear membrane in three-fourths of the cells.

Basolateral membranes, mitochondria, nuclei, and the cytosol did not contain IF in any of the biopsies. The Golgi apparatus was not identified as containing IF after the 3-min biopsies.

DISCUSSION

By localizing IF in fundic biopsies obtained sequentially before and after pentagastrin stimulation, we have been able to delin-

eate a set of intracellular events that occur during IF secretion in man. (a) In the resting stomach, IF is localized to tubulovesicles scattered through the cytoplasm of parietal cells; (b) during the first 8 min after stimulation, the movement of these tubulovesicles with IF to a pericanalicular location takes place; (c) between 8 and 30 min, IF is found on many secretory canalicular and apical microvilli coincident with the peak of IF measured in gastric secretions; (d) from 30 to 60 min, when IF secretion has declined, tubulovesicles with IF are diminished; (e) beginning 15 min after stimulation, IF appears on RER and on perinuclear membrane; and (f) by 60 min, parietal cells with nonstimulated morphology and IF within scattered tubulovesicles reappear. All IF was found to be associated with (bound to, or part of) specific membranous structures.

The observed events suggest that tubulovesicular IF is secreted in response to stimulation by a secretagogue and, when tubulovesicles with IF are exhausted, IF secretion ceases. As tubulovesicles with IF are depleted, synthesis of IF is initiated in the perinuclear space and RER. Whether or not this newly synthesized IF can be secreted in response to stimulation without first associating with tubulovesicles remains to be established. In contrast to parathormone secretion (25), in which newly synthesized hormone may be released before the stored form, it is likely that the storage form of IF is the source of secreted IF during the 15–30 min following stimulation. In addition, the failure of continued secretion of IF during continuous stimulation (5, 8) suggests that the association of IF
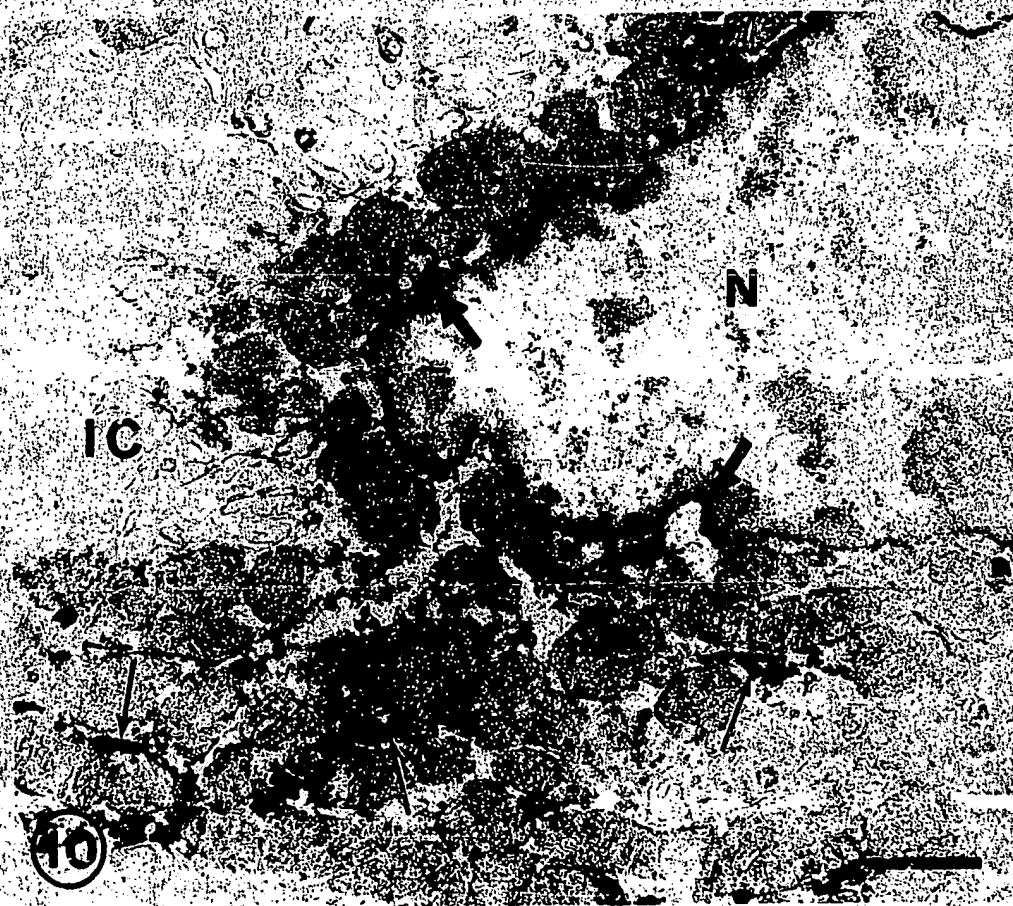

FIGURE 10 Section from 45-min poststimulation biopsy, reacted with antihuman IF. RER interspersed between mitochondria (small arrows) and the perinuclear membrane (large arrow) stain prominently for IF. Minimal microvillar and no tubulovesicular staining is present. N, nucleus. ×15,000, Bar, 1 μm.
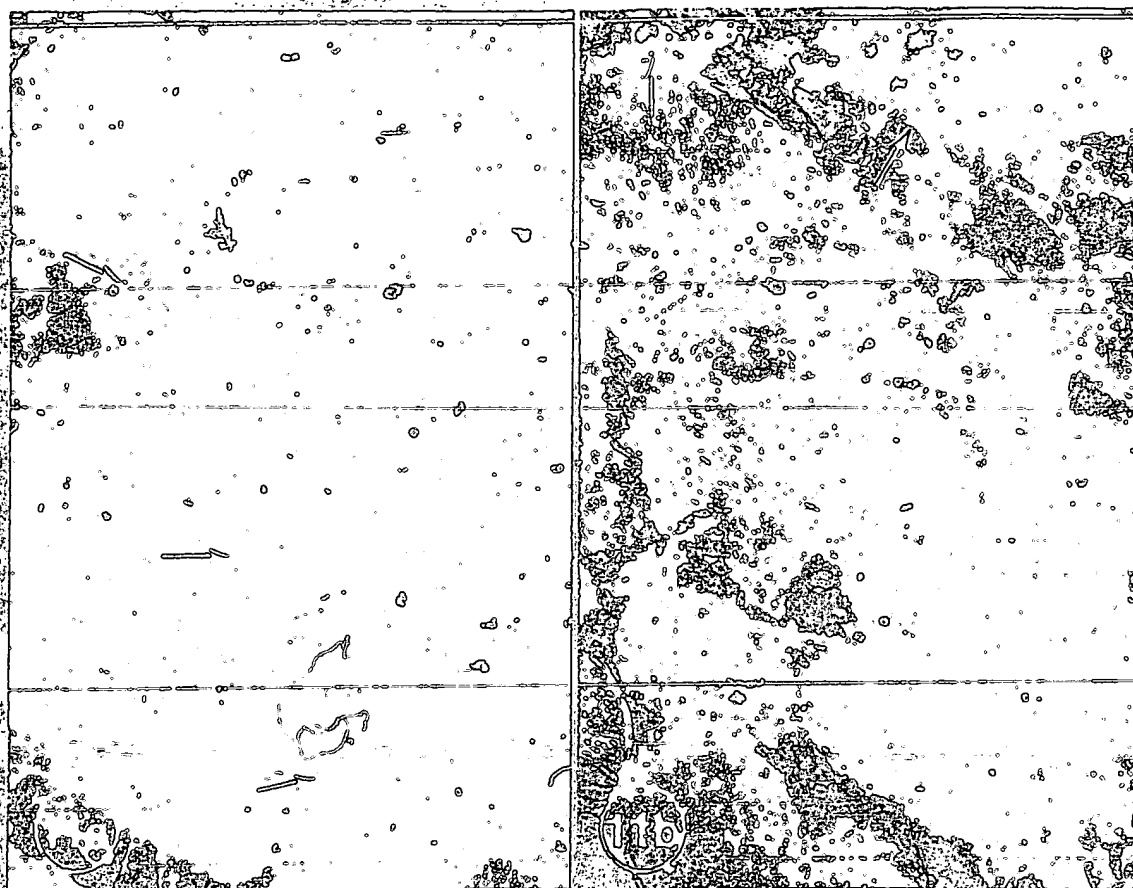
FIGURE 11 Counterstained sections from 45-min poststimulation biopsy that had been reacted with antihuman IF + IF and antihuman IF. (a) Polyribosomes (arrows) are visible on RER in control section. (b) Polyribosomes are obscured on stained RER (large arrows), but these structures are clearly distinguishable from the smooth-membraned tubulovesicles (small arrows). ×44,000 Bar, 0.3 μm.

with tubulovesicles is a prerequisite for secretion. This interpretation favors a wash-out (4) rather than an acid inhibition hypothesis (10).

These changes in the total quantity of tubulovesicles after initiating acid secretion are well known (11–14). There was no attempt to determine what percentage of the tubulovesicles (or any other organelle) within a given cell were associated with IF, because many unstained organelles are not visible with our noncounterstained method. Counterstaining is not used because it obscures specific immunocytochemical staining. Therefore, the subcellular changes noted in the postpentagastrin biopsies apply only to stained organelles.

Presently, two hypotheses have been offered to explain the morphologic changes from the nonstimulated parietal cell with diffuse tubulovesicles and small intracellular canaliculi to a stimulated cell with few tubulovesicles and expanded secretory canaliculi (11–14). On the basis of transmission EM studies, Forte et al. (15) have suggested that tubulovesicles translocate, fuse, incorporate into the intracellular canaliculi, and form secretory canaliculi. Berlindh et al. (26), using dye incorporation studies, have suggested that tubulovesicles are connected to the intracellular canaliculi in the resting state, although they appear as separate membranous structures in ultrathin sections. Upon stimulation, primary osmotic changes expand the intracellular canaliculi and tubulovesicles to form secretory canaliculi. The results of the present study support the translocation-fusion hypothesis. The initial event observed after stimulation is the movement of scattered tubulovesicles with IF to an area

immediately surrounding intracellular canaliculi, at a time (3 and 8 min, Figs. 4 and 5) when there is no evidence of intracellular canalicular expansion. We cannot explain logically this sequence of events on the basis of a primary osmotic change within the intracanalicular system.

The current studies have resulted in information regarding the migration of IF from the intracellular storage site to the apical cell surface. The mechanism involved in the release of IF from the cell surface into the secretory canaliculi could not be ascertained. From these immunoelectronmicroscopic observations, it appears that IF is associated with tubulovesicular and, later, microvillar membranes. Several possible explanations can be made for these findings. The first is that IF is an integral part of these membranes. Alternatively, IF may appear to be an integral part of the membrane as a result of an artifact of the immunoelectronmicroscopic technique, through which IF may become selectively bound to membranes during fixation; nonmembrane-bound IF, as could occur within tubulovesicles, may be washed away because of inadequate fixation, or the peroxidase reaction product may bind selectively to membranes. If IF is in fact an integral part of the membrane, as many cell glycoproteins are, the final step in the secretory process must be either the selective dissociation of IF from the membrane or the release of intact membrane from the cell. However, to conclude that IF is a part of the membrane, studies will be required in which intact vesicles (27) are isolated.

Similarly, the fate of tubulovesicles that are present within microvilli (Fig. 7 b) is unknown. It is possible that they (a) are
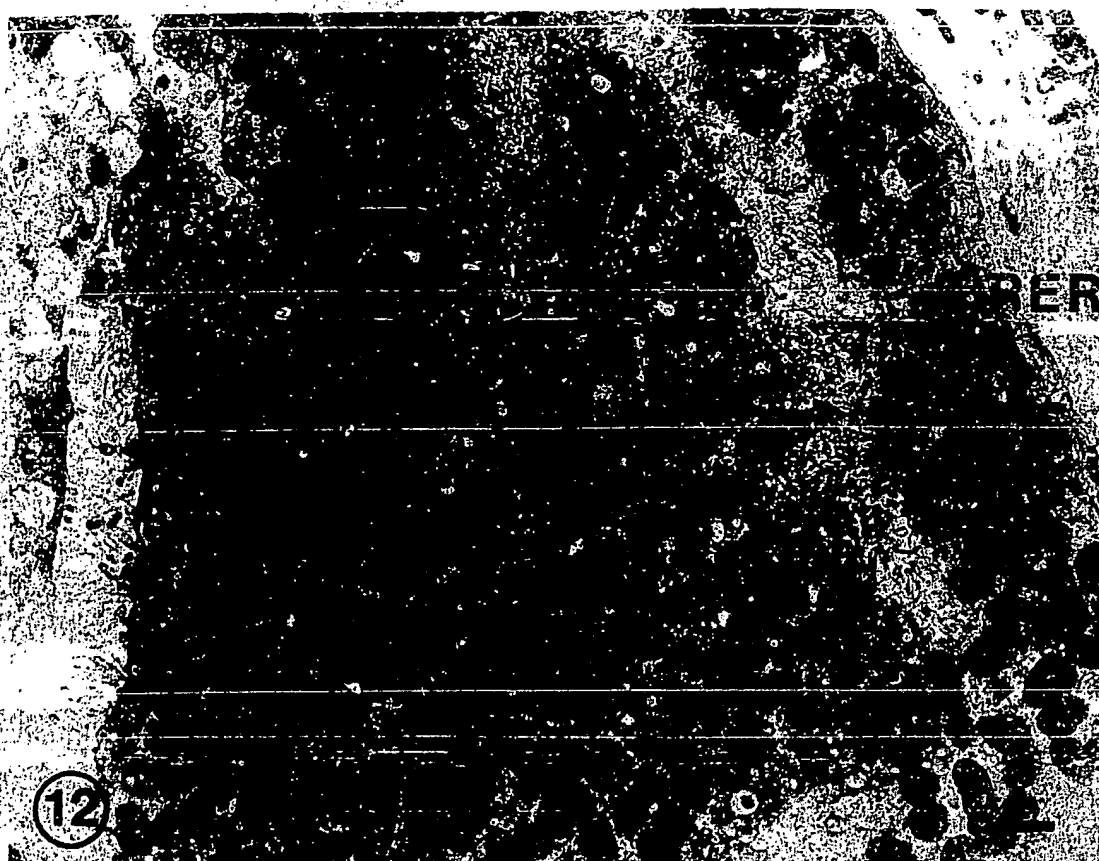
RER

in the process of fusing with microvilli, (b) are a sectioning artifact, or (c) reflect tubulovesicles that will not fuse with microvilli and are available for recycling. Credence is given to the latter possibility by the finding of a pericanalicular tubulo-vesicular structure in direct membrane continuity with an or-ganelle, the multivesicular body (Fig. 9), that may be involved with membrane recycling. That surface membrane associated with IF can be recycled, as well as secreted, seems likely, but whether the recycled IF is degraded or directly made available for secretion cannot be defined from our in vivo study.

Our sequential observations of the intracellular distribution of IF from resting to stimulated and back to resting cells also provide an improved understanding of the steps that lead from IF synthesis to storage. The nonstimulated parietal cell had small amounts of IF on RER and Golgi apparatus that were always confined to the basal portions of the cell (Fig. 3 a, b). Portions of perinuclear membrane also contained IF. Shortly after administration of pentagastrin the distribution of IF in these organelles underwent changes. By 15 min, increased amounts of RER containing IF were interspersed throughout the cytoplasm of 25% of the cells visualized. The perinuclear membrane was most often densely and uniformly stained in these cells. This pattern was seen in essentially 100% of the cells found in biopsies obtained at 45 min (Fig. 10). IF synthe-sis, therefore, appeared to be initiated at the same time that stored IF tubulovesicles were being secreted. From our in vivo observations, we cannot define whether IF synthesis is triggered by the pentagastrin or by the actual secretory process itself. The inability of light microscopic immunohistochemistry to

detect differences in the cellular content of IF before, during, and after secretion exists because of one IF containing structure (tubulovesicles) being replaced by another (RER).

The steps leading from IF synthesis in RER and perinuclear membrane to the formation of tubulovesicles with IF were not ascertained in our study. Although studies in several other cell systems (28, 29) have provided evidence that under steady-state conditions the Golgi apparatus is an important intermediary in the production of surface membrane proteins, we could not document the presence of IF in the Golgi complex during the recovery stage. Caution must always be maintained, however, in the interpretation of the lack of immunogenicity in any immunohistochemical study, because this can be due to a methodologic artifact (30). It is possible, for instance, that penetration of the antibody to the interior of Golgi saccules may be poor, that the antigenic site on the IF molecule may be hidden during transportation through the Golgi complex, that IF is more soluble when it is in the Golgi complex and not fixed to that site or that the events occurred too rapidly to be observed with the frequency of biopsies that were obtained. However, because IF could be localized to the Golgi apparatus of nonstimulated cells (Fig. 3 b), it is unlikely that the antigenic site was masked or that the antibody inadequately penetrated.

If the Golgi apparatus is not involved in the formation of new storage tubulovesicles in the poststimulated parietal cell, we might speculate that tubulovesicles bud directly from RER. A similar mechanism has been suggested for the synthesis of smooth endoplasmic reticulum from RER during hepatic em-bryogenesis (31). Clarification of mechanisms will require in

vitro studies of parietal cells.

In summary, using a well-characterized immunocytochemical protocol we have been able to document a reproducible sequence of intracellular events during human IF secretion. Secretion of this protein from the parietal cell requires membrane-associated vesicular transport with no observable role for the Golgi apparatus or secretory granules. Whether this represents a unique circumstance in a highly specialized cell type, or is an alternative mechanism for secretion of proteins synthesized within cells, can be determined only through studies in other organ/cell systems.

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